Clinical Evaluation of the Uni-Yeast-Tek System for Rapid Presumptive Identification of Medically Important Yeasts

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Received for publication 22 August 1977

The results of over 400 tests for identification of clinical yeast isolates as to species using the Uni-Yeast-Tek (UYT) system in comparison with a more conventional system are reported. The conventional system utilized a total of 23 individual tests, including both fermentation and assimilation tests, whereas the UYT system included only 11 separate tests. In the initial phase of the study, coded unknown isolates were evaluated by each of two technologists using both methods independently. After this initial evaluation, the two methods were used in parallel for routine testing of yeast isolates as they were obtained from clinical specimens. A further evaluation of the UYT system was carried out by retrospectively analyzing the species reported from a clinical mycology laboratory during two separate time periods in which different approaches to yeast identification were employed. A total of 92% of the isolates tested with the UYT system were correctly reported within 72 h, 96% were correctly named after 1 week of incubation, and 97% were correctly reported after 2 weeks of incubation of UYT plates at 30°C when results of the two phases of the study were analyzed together. With the conventional system, 88% of the isolates were correctly reported at 72 h, 96% at 1 week, and 98% after 2 weeks of incubation of biochemical tests. Retrospective analysis of laboratory records revealed no major changes in species reported after adoption of the UYT system for routine testing of clinical isolates. The data presented in this report suggest that the UYT system can be expected to yield rapid presumptive identification of clinical yeast isolates with reasonable confidence when certain minor limitations that are discussed in the text are taken into account.

The increasing number of serious and often fatal yeast infections currently being observed in immunosuppressed patients has encouraged the development of more rapid yeast identification methods. Identification of yeasts isolated from clinical materials as to species increases our understanding of the pathogenic potential of various yeast species in compromised hosts, aids in the interpretation of serological tests, assists with the selection of newer antifungal drugs such as 5-fluorocytosine for treatment of yeast infections, and allows a definitive diagnosis to be made in patients suffering from yeast infections. Isolation of the same yeast species from multiple specimens from a patient suggests at least extensive colonization of the patient with the species in question and should not be regarded lightly.

Many clinical laboratory workers are reluctant to attempt extensive characterization of yeast species because traditional yeast identification techniques (17, 19) are regarded as being too complex and too time-consuming to be practical. In addition, the lack of commercial availability of some of the media needed for carrying out carbohydrate and nitrate assimilation tests, which are important tools used by yeast taxonomists, has in the past discouraged the routine use of such tests. However, in the past few years products that shorten the time required for presumptive identification of yeasts have been evaluated (5-7, 9, 10, 12-14), and the commercial availability of some of these products encourages more extensive biochemical characterization of yeast isolates. Still, these newer methods must be shown to produce reliable results before they can be accepted for routine use, and they will not substitute for an adequate understanding of the biochemical reactions utilized in the various biochemical tests. They merely make yeast identification more convenient for well-trained technologists who are familiar with standard procedures and who are aware of the limitations of those techniques.

The Corning Uni-Yeast-Tek (UYT) system is one of the newer commercially available products that combines several of the media required for yeast identification into a single device. This type of product permits inoculation of several biochemical tests at the same time while reducing the amount of space required for storage of the media and eliminating the necessity of preparing the test media in individual laboratories. The UYT system has been evaluated previously by Bowman and Ahearn (5, 6), who reported that 99% of the ioslates they tested were correctly identified with it within the 6-day incubation time recommended by the manufacturer.

It is the purpose of this paper to describe experiments leading to the conclusion that the UYT system provides a reliable approach to rapid presumptive identification of clinical yeast isolates within 72 h from the time of inoculation when UYT plates are incubated at 30°C. Although not rated to accomplish this task in less than 6 days by the manufacturer, we have evaluated the performance of the UYT system for rapid presumptive identification because of the need to make mycology laboratory results available to physicians as rapidly as practical. In the present paper, studies previously reported by Bowman and Ahearn (5, 6), using stock isolates of medically important yeasts, are extended by (i) incorporating the UYT system into the work routine of a clinical mycology laboratory in parallel with a more conventional system, (ii) incubating UYT test plates at 30°C, and (iii) recording results after 72 h of incubation.

MATERIALS AND METHODS

Study design. The majority of isolates used for the study were recent clinical isolates obtained from three different hospital laboratories; however, stock reference strains obtained from the American Type Culture Collection and isolates recovered from soil specimens were also included. In the initial phase of the study, coded isolates maintained as stock cultures were streaked on Sabouraud glucose agar, incubated at 30°C for 48 h, and then given to each of two technologists to identify by using the UYT system and a conventional procedure. Each technologist identified at least 100 isolates in this initial phase of the study, and each identified most of the same isolates, although at different times and coded with different numbers. In the second phase of the study, the two methods were used in parallel for routine testing of isolates obtained from clinical specimens in the diagnostic mycology laboratory of Baylor University Medical Center. These isolates were selected for incorporation into the study on the basis of the results of a germ tube test so that isolates of Candida albicans would comprise no more than 25% of those tested although that species is isolated from a much higher percentage of clinical specimens. Clinical isolates were restreaked for isolation on Sabouraud glucose agar and incubated at 30°C for 48 h before being tested further. In one sense, then, these isolates were also coded isolates since their final identification was not known at the time that they were selected for the study.

As much as possible, all biochemical tests were inoculated at the same time by using a suspension of each isolate prepared by dispersing a single colony in 5.0 ml of sterile distilled water. Each tubed medium was inoculated with 0.1 ml of this suspension while each well of the UYT plate was inoculated with 1 drop of the suspension by using a sterile Pasteur pipette. All assimilation tests were incubated at 30° C, and results were recorded after 72 h, 1 week, and 2 weeks of incubation.

When preliminary results of the study became available, the UYT system was adopted for routine use for identifying clinical isolates as to species. As a further evaluation of the reliability of results obtained with this system, a retrospective analysis of species reported during the 6-month period before its adoption and of the species reported during the year since it has been used routinely was carried out. A large shift in the overall percentage of any one species reported would reflect significant misidentification or, perhaps. increased reliability obtained with the UYT system when random fluctuations in the distribution of species isolated are taken into account. For this analysis. reports recorded in the laboratory logbook during the period from January 1976 through June 1976 were compared with reports logged from July 1976 through June 1977.

Identification methods. The conventional method utilized for comparison with the UYT system included a total of 23 separate tests consisting of (i) a germ tube test; (ii) an India ink mount to detect capsules; (iii) morphology on cornmeal agar with Tween 80; (iv) urease reaction; (v) assimilation of glucose, maltose, sucrose, lactose, galactose, melibiose, cellobiose, inositol, xylose, raffinose, trehalose, and dulcitol; (vi) fermentation of glucose, maltose, sucrose, and lactose; (vii) assimilation of KNO3; (viii) growth at 37°C; and (ix) color of colonies on birdseed agar. This conventional system has been utilized for some time and has been shown to produce reliable identifications of yeasts isolated from clinical materials in our laboratory. The procedures followed provide an adequate testing of the morphological and biochemical properties of clinical yeast isolates, although rare isolates do require additional tests for exact identification. The battery of 45 or more tests utilized for definitive taxonomic studies (11, 19) is too time-consuming to be practical, and experience has shown that the battery of tests included in our conventional system is a reasonable compromise between a definitive taxonomic system and a system in which only "yeasts isolated" is reported.

The UYT system includes 11 tests consisting of (i) a germ tube test; (ii) morphology on cornmeal agar; (iii) urease reaction; (iv) assimilation of sucrose, lactose, maltose, raffinose, cellobiose, soluble starch, and trehalose; and (v) assimilation of KNO₃. Identifications were made independently with the two methods by using a standard reference (11) for the conventional system and the "Logic Wheel" supplied by the manufacturer for UYT.

(i) Urease reaction, India ink, morphology, and growth at 37°C. Conventional techniques for inoculation, incubation, and interpretation were utilized for all of these techniques (15, 17), with the exception that media for the urease reaction and for morphological studies were different. Christiansen urea agar and cornmeal agar purchased in prepared form from Randolph Biologicals were utilized for urease and morphology, respectively, in the conventional system. The urea medium and cornmeal agar supplied with the UYT system were utilized for the urease reaction and for morphological studies with that system. Tests for capsules by India ink and for growth at 37°C were utilized for the conventional system only.

(ii) Germ tube test. For the germ tube test with the conventional system, a slightly turbid suspension of a single yeast colony was made in 0.5 ml of fetal calf serum (Flow Laboratories, Inc.), using a sterile Pasteur pipette essentially as described by Ahearn (2, 3). The suspension was then incubated at 37°C for 2 h, and a portion was examined microscopically for the production of short hyphal outgrowths without constrictions from yeast cells that are commonly called germ tubes. For the UYT system the same procedure was followed except that the glucose beef extract medium supplied by the manufacturer was used.

(iii) Carbohydrate assimilation tests. For the conventional system, modified Wickerham agar slants purchased from Randolph Biologicals were utilized for testing for assimilation of the 12 carbohydrates listed earlier. The medium contained bromocresol purple indicator, and a positive test was recorded when acidification of the medium changed the indicator from purple to yellow. In a previous evaluation of this medium, the change of the indicator was shown to be synonymous with growth (1). The UYT system included only seven carbohydrates, but the medium also contained bromocresol purple indicator, and a positive assimilation test was recorded when the indicator changed from purple to yellow. The results of all assimilation tests were verified by using a modified auxanographic technique (15) in parallel with the other assimilation tests.

(iv) Carbohydate fermentation. Fermentation of glucose, maltose, sucrose, and lactose was used in the conventional system only. These tests were carried out essentially as recommended by Haley (8), using beef extract broth as the basal medium. After inoculation, the broth was covered with a Vaspar seal and incubated at 37°C. A positive test was recorded when gas was detected in either the Durham tube inserts or underneath the Vaspar seal.

(v) Nitrate assimilation. For the conventional system, assimilation of potassium nitrate was detected with an agar slant medium prepared according to the formulation given by Vera and Dumoff (18) with the addition of 1.5% Noble agar (Difco Laboratories). This medium did not contain an indicator, and for each test a negative control slant containing only yeast carbon base and a positive growth control slant containing peptone as the nitrogen source were each inoculated along with the test slant containing KNO₃ as the nitrogen source. A positive test was recorded when growth on the KNO₃ slant was equivalent to growth on the peptone slant.

The UYT nitrate test contained bromothymol blue indicator, and a postive test was recorded when growth on the medium was sufficient to produce an alkaline pH changing the indicator from yellow to green or blue.

(iv) Birdseed agar. In the conventional system,

birdseed (Guizotia abyssinica) agar was used as an aid in the identification of Cryptococcus neoformans. The birdseed agar used was purchased from Randolph Biologicals. The medium was made up according to the original formulation described by Staib and Senska (16) except that the G. abyssinica extract was filtered to remove the seed hulls before it was incorporated into the basal medium. The medium also contained chloramphenicol to inhibit bacterial growth. Colonies of test yeasts were streaked for isolation on the medium, incubated at 30°C, and observed for the production of brown colonies. Those yeasts producing brown colonies were presumptively identified as Cr. neoformans.

RESULTS

As shown in Table 1, 89% of the coded unknown isolates were correctly reported in 72 h by using the UYT system, and 94% were correctly reported after 1 week of incubation at 30°C. By comparison, the percentage of coded isolates correctly reported with the conventional system was somewhat lower at 72 h and 3% greater at 1 week. The only major misidentification reported with the UYT system occurred with isolates of Cr. uniguttulatus, which were reported as Cr. neoformans. The correct biochemical reactions were given by each of the Cr. uniguttulatus isolates; however, the number of tests available with the UYT system does not permit differentiation of Cr. uniguttulatus and Cr. neoformans. The maximum variation of 8% between results reported by the two technologists occurred with the conventional procedure at the 72-h reading. With the UYT system at all time periods, the results reported by the two technologists were in close agreement.

Table 2 shows results reported with single isolates of six species that are only infrequently encountered in clinical specimens. Four of the six species were incorrectly named, but with C. macedoniensis and C. utilis both technologists recognized that additional tests would be necessary for correct identification as to species. Data obtained with these isolates are not included in the tabulation of results in Table 1, but are presented as a matter of interest only. Again, the correct biochemical reactions were obtained with each isolate; however, the number of biochemical tests available caused some misidentifications, and it should be noted that, with the exception of Trichosporon capitatum which was correctly identified with the conventional system, the results reported with the two methods were the same.

Table 3 shows the data obtained when both methods were used at the same time for testing clinical isolates. In this phase of the study, the percentage of species correctly reported was slightly greater at all time periods when the

Organism	No. of isolates tested _	% Correctly reported by:				
		Conventional method		UYT		
		72 h	1 week	72 h	1 week	
Candida albicans	17	94	100	100		
C. guilliermondii	19	84	100	84	95	
C. krusei	18	94	94	94	100	
C. parapsilosis	20	80	100	95	100	
C. pseudotropicalis	8	100		100		
C. stellatoidea	8	88	100	88	100	
C. tropicalis	12	83	100	100		
Cryptococcus albidus	15	67	87	80	93	
Cr. laurentii	11	100		100		
Cr. neoformans	19	74	95	100		
Cr. terreus	10	20	80	70	80	
Cr. uniguttulatus	9	67	100	0	0	
Rhodotorula glutinis	4	100		100		
R. rubra	3	100		100		
Saccharomyces	3	100		100		
Torulopsis glabrata	20	100		95	100	
Trichosporon cutaneum	10	100		100		
Total	206	84	97	89	94	

TABLE 1.	Results	obtained	with	coded	isolates
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TABLE 2.	Misidentifications obtained with unusual
i	solates by using the UYT system

Species	Reported as:
Candida aeseri	C. parapsilosis
C. lipolytica	C. krusei
C. macedoniensis	Need further tests
C. utilis	Need further tests
C. zeylanoides	Torulopsis glabrata
Trichosporon capitatum	

UYT system was used. When results of the two phases of the study were combined, it was found that 92% of the isolates tested were correctly named after 72 h of incubation, 96% after 1 week of incubation, and 97% after 2 weeks of incubation with the UYT system. These results are essentially equivalent to those obtained with the conventional system. At 72 h, 4% more isolates were correctly named with the UYT system; at 1 week, the percentages were the same; and after 2 weeks of incubation, 1% of all the isolates tested in both phases of the study that were misidentified with the UYT system were correctly named with the conventional system.

As shown in Table 3, one isolate of *C. albicans* was incorrectly named by the UYT system. This isolate failed to produce either germ tubes or chlamydospores; however, it produced biochemical reactions typical of *C. albicans*. Closer in-

spection of the results on the UYT plate revealed negative cellobiose assimilation that might have suggested the correct identification; however, the Logic Wheel routinely identifies such isolates as *C. tropicalis*. The one isolate of *T. candida* encountered among the clinical isolates could not be identified with the UYT system, but the technologist was alerted to carry out additional tests by examining the assimilation tests on the UYT plate.

As the second phase of the study progressed, it became apparent that tests for cellobiose assimilation with clinical isolates of C. tropicalis were quite variable. All stock C. tropicalis isolates tested earlier in the initial phase of the study had given positive tests for assimilation of cellobiose with both methods; however, recent clinical isolates of that species behaved in an unpredictable fashion with regard to cellobiose assimilation. A closer inspection of cellobiose assimilation by 67 clinical isolates compatible with C. tropicalis on the basis of all other morphological and biochemical characteristics tested revealed that 52 (78%) were positive for cellobiose assimilation with the UYT system, whereas 34 (51%) were positive with the agar slant medium. Seven (10%) were negative by both methods. It should be pointed out that in spite of variations in the results of cellobiose

		% Correctly reported by:			
Organism	No. of isolates tested	Conventional method		UYT	
		72 h	1 week	72 h	1 week
Candida albicans	36	94	100	94	98
C. guilliermondii	2	0	0ª	100	
C. krusei	6	100		100	
C. parapsilosis	16	56	81ª	81	100
C. pseudotropicalis	3	67	67ª	100	
C. stellatoidea	2	100		100	
C. tropicalis	67	97	97ª	98	100
Cryptococcus albidus	1	0	100	0	100
Cr. neoformans	6	100		100	
Geotrichum species	1	100		100	
Saccharomyces	1	100		100	
Torulopsis candida	1	0	100	Need further tests	Need further test
T. glabrata	56	98	98	98	100
Trichosporon cutaneum	5	100		100	
Total	203	92	96	96	99

 TABLE 3. Results obtained with clinical isolates

^a 100% at 2 weeks.

assimilation, correct identification of all clinical *C. tropicalis* isolates as to species was achieved with each of the methods. This was accomplished by relying on the results of sucrose fermentation along with other morphological and biochemical tests with the conventional method. Only a single isolate of the 67 tested failed to ferment sucrose. This isolate did, however, assimilate cellobiose and was correctly named.

With the UYT system, the Logic Wheel names any isolate that is germ tube negative, urease negative, lactose negative, maltose positive, raffinose negative, and positive on soluble starch as *C. tropicalis.* Isolates of *C. albicans* that are germ tube negative will give this same sequence of biochemical reactions; therefore, careful inspection of the morphology of such isolates on cornmeal agar and of the results of the cellobiose assimilation test is necessary for correct identification.

Retrospective comparison of species reported during the time period in which the conventional method was used with species reported after the adoption of the UYT system as the method of choice for studying clinical yeast isolates revealed only minor shifts in percentages of species reported. During the year after the adoption of the UYT system, the percentages of *C. krusei* and *C. parapsilosis* increased by 1%, whereas the percentage of *C. albicans* declined slightly and the percentages of *C. tropicalis* and *T. glabrata* reported remained the same. A total of 27 isolates of *Cr. neoformans* were correctly identified as to species with the UYT system used in combination with birdseed agar during this time period.

To accomplish the stated goals of this study, critical evaluation of each component in the UYT test system was necessary. The corning glucose-beef extract medium used to carry out germ tube tests gave results that agreed almost completely with results obtained with fetal bovine serum in parallel tests. However, the number of germ tubes observed was usually greater in fetal bovine serum. The urease test gave 100% agreement with Christiansen urea agar used in comparative tests, and morphology on the cornmeal agar agreed with morphology observed on cornmeal Tween 80 agar used for comparison. The greater rate of positive assimilation of cellobiose observed on the UYT plate has already been described. Assimilation of other carbohydrates agreed very closely with results obtained with the comparative method. Delayed assimilation of cellobiose and trehalose was observed on all media, and these delayed reactions accounted for the majority of misidentifications recorded at 72 h. The nitrate test on the UYT plate was found to be very easy to interpret and gave more reliable results than did the agar slant method.

DISCUSSION

The reliability of results obtained with the UYT system is surprising in view of the relatively few tests that are included with it. The overall precentage of correct identifications obtained with the UYT system reported here is somewhat lower than that reported by Bowman and Ahearn (5, 6); however, the results obtained when the UYT system was used in parallel with a conventional system for identifying clinical isolates as to species (Table 3) were essentially the same as those reported by previous investigators. The lower percentage of correct identifications obtained with the UYT system using coded isolates can be attributed to the inclusion of species in the initial phase of the study that are only occasionally encountered in clinical specimens, such as Cr. uniguttulatus. Designation of Cr. uniguttulatus as Cr. neoformans was the major misidentification encountered with the UYT system, and the impact of that misidentification on the routine operation of a clinical mycology laboratory would be expected to be guite minimal. It is important, however, to be able to differentiate the two species because Cr. uniguttulatus has not been reported as a cause of human disease up to the present time. Administration of antifungal drugs to patients from whom Cr. uniguttulatus is isolated is considered to be inappropriate in light of our present knowledge.

Cr. neoformans can easily be differentiated from Cr. uniguttulatus by carrying out tests for the production of brown colonies on birdseed agar and for the ability to grow at 37°C in addition to those morphological and biochemical tests included in the UYT system. Alternatively, an additional test for the assimilation of dulcitol could be utilized. Cr. neoformans would be expected to give positive results for all three of the aforementioned tests, whereas Cr. uniguttulatus gives negative results. In our laboratory, all specimens submitted for the isolation of fungi are routinely plated on birdseed agar and on media that are incubated at 37°C, so that by the time yeast colonies are obtained on these primary isolation media, the ability to produce brown colonies on birdseed agar and the ability to grow at 37°C have already been determined. In that setting, the battery of biochemical tests in the UYT plate is adequate for confirming a presumptive identification of Cr. neoformans made on the basis of the appearance of yeast colonies on the primary isolation media. As judged from the results obtained in this study and from experience gained by using birdseed agar as a primary plating medium for over 3 years, the production of brown colonies on that medium is a reliable criterion for presumptively identifying a yeast isolate as Cr. neoformans. Of all the tests carried out on birdseed agar in our laboratory to

date, only a single isolate of *Cr. albidus* obtained from soil has given a false-positive result, whereas all valid isolates of *Cr. neoformans* have produced brown colonies.

Results obtained with tests for cellobiose assimilation by C. tropicalis are quite interesting in that they illustrate strain variability with regard to this property as the species C. tropicalis is presently defined. The yeast isolates that include germ tube-negative and chlamydosporenegative C. albicans, C. tropicalis, and C. parapsilosis have been, in our experience, more difficult to identify exactly as to species than are other isolates of Candida species. Inclusion of soluble starch in the UYT plate has proven to be an advantage for differentiation of C. parapsilosis from the other two species. C. parapsilosis isolates were usually identified more quickly with the UYT system than with conventional methods in the experiments reported here (Table 3). Differentiation of C. tropicalis from those unusual C. albicans isolates that neither form germ tubes nor produce chlamydospores depends upon tests for cellobiose assimilation and sucrose fermentation in most conventional systems. As noted in this study, 10% of the C. tropicalis clinical isolates failed to assimilate cellobiose by either of the methods used. Isolates of C. tropicalis that gave negative results for sucrose fermentation have recently been reported by Ahearn et al. (4), and C. albicans isolates that gave positive results for sucrose fermentation were recently reported bv D'Amato et al. (Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C97, p. 51). All of these findings taken together suggest that the distinction between C. albicans and C. tropicalis may not be as great as once thought. This may be a reflection of the variability of the two species as presently defined, or, because these variabilities are more apparent with recent clinical isolates than with stock isolates, it may be a reflection of selective pressures on genetic control of alphaglucoside metabolism of C. tropicalis occasioned by growth in human tissues. These phenomena might also be an indication of selective pressures exerted by certain drugs that are used to treat human diseases.

The Logic Wheel provided with the UYT system as an aid to making identifications offers the advantage of providing a species name each time it is used. However, careful inspection of the characteristics listed in tabular form as "Range of Expected Reactions" on the reverse of the Logic Wheel is necessary for accurate interpretation of biocheimal reactions on the wheel plate. In this study, some minor discrepancies between observed results and those listed in the Logic Wheel table were noted. For example, the variability of cellobiose assimilation by C. tropicalis has already been pointed out. The Logic Wheel table also lists Cr. albidus as being positive for lactose assimilation, which is correct for the Cr. albidus variety, but is incorrect for the Cr. diffluens variant. Expansion of the table to include both varieties of Cr. albidus would eliminate this minor point of confusion. Further expansion of the table to include Cr. uniguttulatus along with a recommendation for carrying out those additional tests necessary for differentiating this species from Cr. neoformans would further enhance the value of the information available in the table.

Identifications obtained with the UYT system have been labeled as "presumptive" in this report as a conservative approach to designating species when so few biochemical tests are utilized. However, the data obtained in this study suggest that more definitive identifications can be obtained only by using a battery of tests different from those employed in the conventional system used for comparison with the UYT system. This applies only to isolates that are commonly encountered in clinical specimens. Some isolates from food, water, soil, and other natural sources can be more easily identified as to species with the conventional system employed here; however, definitive taxonomic identification of yeast species as found in standard reference works (17, 19) can be obtained only by using the extensive battery of tests commonly employed by yeast taxonomists.

When the limitations discussed above are taken into account, the UYT system can be expected to produce presumptive identifications of clinical yeast isolates with reasonable confidence within 72 h when the isolates are incubated at 30°C. Results of over 400 yeast identification tests obtained in this study and the results of a retrospective analysis of species recorded by using the UYT system for routine testing of clinical isolates support this conclusion.

ACKNOWLEDGMENTS

This research was supported by a grant of supplies and materials from Corning Medical Diagnostics and by the Special Fund for Mycology Research and Education of Baylor University Medical Center.

We express our appreciation to Robin Hunter, Debbie Perrault, and Mary D'Anton for technical assistance and to Janelle Davis for typing and revising the manuscript. Many of the cultures used for this report were contributed by Richard Clark, Geoffery Land, and Glenn Roberts.

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